AD_____

AWARD NUMBER: W81XWH-05-1-0055

TITLE: Dendritic Cell-Based Genetic Immunotherapy for Ovarian Cancer

PRINCIPAL INVESTIGATOR: James M. Mathis, Ph.D.

CONTRACTING ORGANIZATION: Louisiana State University
Shreveport, Louisiana 71130

REPORT DATE: December 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-12-2005	Annual	1 Dec 2004 – 30 Nov 2005

4. TITLE AND SUBTITLE

Dendritic Cell-Based Genetic Immunotherapy for Ovarian Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0055

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

James M. Mathis, Ph.D.

E-Mail: jmathi@lsuhsc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Louisiana State University
Shreveport, Louisiana 71130

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Adenovirus (Ad)-mediated transduction of dendritic cells (DCs) is inefficient because of the lack of the primary Ad receptor, CAR. CD40 is a surface marker expressed by DCs that plays a crucial role in their maturation and subsequent stimulation of T cells. DC infection with Ad targeted to the CD40 results in increased gene transfer. Cells transduced with CD40-targeted Ad5-SV40-TAg vector showed increased expression of transgene and expression of co-stimulatory molecules at 48 hours post-infection compared to cells transduced with untargeted Ad5-SV40-TAg vector. We demonstrated that CD40-targeted gene transfer promotes DC maturation with induction of a complex signaling cascade accompanied by characteristic changes in cytokine production. These results demonstrate that DCs can be successfully transduced using a CD40 targeted adenoviral vector and that transduced DCs show activation.

15. SUBJECT TERMS
ovarian cancer; gene therapy; dendritic cells; adenovirus; cd40; tumor antigen; vaccination; targeting; ctl response; antigen presenting cell; syngeneic tumor model; immunization; preclinical

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	15	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Introduction

Dendritic cells (DCs) capture, process and present antigens in association with MHC class I and class II molecules to naive CD8+ cytotoxic and CD4+ helper T cells. Through this, specific cytotoxic T cells are activated, and recognize a target cell and kill it. This study was to determine the transduction efficiency of DCs using a CD40-targeted adenoviral vector expressing a tumor antigen. Recently, we characterized a new model using a mouse ovarian carcinoma cell line (MOVCAR) that expresses the SV40 large T-Ag and forms tumors in syngeneic mice. The SV40 large T-Ag is highly immunogenic, inducing both antibody and cytotoxic T lymphocyte (CTL) responses. Since this antigen is synthesized in MOVCAR cells, the SV40 large T-Ag is an attractive candidate as a model system for the development of a DC-targeted cancer vaccine. We hypothesize that transduction of DCs in vitro using a CD40-targeted Ad5 vector expressing SV40 T-Ag (Ad5-SV40-TAg) will result in a high level of transgene expression, and be effective in inducing an antigen-specific CTL response. To target Ad5-SV40-TAg to DCs, we utilized a recombinant adapter protein consisting of extracellular portion of the native adenovirus receptor, CAR, fused to a trimerization motif from T4 fibritin protein, and linked to the extracellular domain of the mouse CD40 ligand.

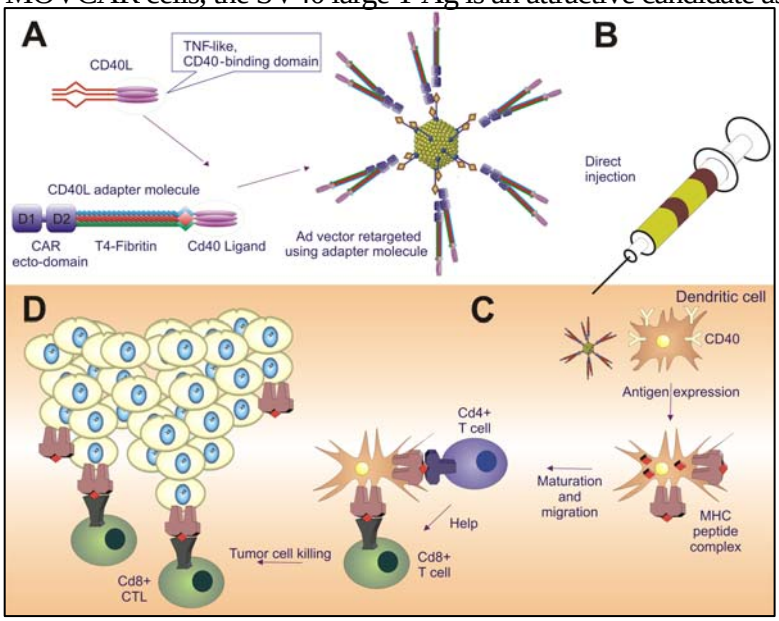


Figure 1. Targeting of Ad5 Vector to CD40 with TNF-Like Domain of CD40L Provides Flexible Platform for DC Transduction. The development of an in vivo approach based on DC vaccination without isolation and culturing of DCs ex vivo would be clinically significant. However, a critical component of in vivo transduction is efficient targeting of the vector to DCs without perturbation of DC function. To this end, we generated Ad vector systems that specifically target human and mouse DCs via the CD40 receptor using CAR-CD40L bi-specific adapter molecules **(A)** and showed that CD40-targeted Ads efficiently transduce DCs in vitro without interfering with DC function. We evaluated a dendritic cell-targeted Ad vaccine expressing the simian virus 40 (SV40) large T antigen (T-Ag) in a mouse model of ovarian cancer. We hypothesize that immunization of DCs **(B)** with the SV40 T-Ag will be effective in inducing antigen-specific cytotoxic T-lymphocyte (CTL) responses **(C)**, and suppress the growth of ovarian tumor cells expressing the SV40 T-Ag **(D)**.

Body

In Task 1 we investigated whether transduction of DCs in vitro with a CD40-targeted Ad vector expressing the SV40 T-Ag [Ad5-(SV40 T-Ag)-CFm40L] will result in a high level of transgene expression, and be effective in inducing an antigen-specific CTL response in vivo. As shown by Western blot analysis (Figure 2A), expression of the SV40 T-Ag transgene occurred only in cells that were transduced with the CD40-targeted adenoviral vector (Ad5-SV40 T-Ag). Thus, DCs were efficiently transduced using a CD40 targeted adenoviral vector, while an untargeted adenoviral vector poorly transduced DCs. As shown in Figure 2B, DCs were treated with untargeted and CD40-targeted Ad5-SV40-TAg from 0 to 48 hours, and Western blot analysis was used to determine the level of expression of SV40 T-Ag in the transduced DCs. Cells transduced with CD40-targeted Ad5-SV40-TAg vector showed increased expression of transgene and expression of co-stimulatory molecules at 48 hours post-infection compared to cells transduced with untargeted Ad5-SV40-TAg vector. These results demonstrate that DCs

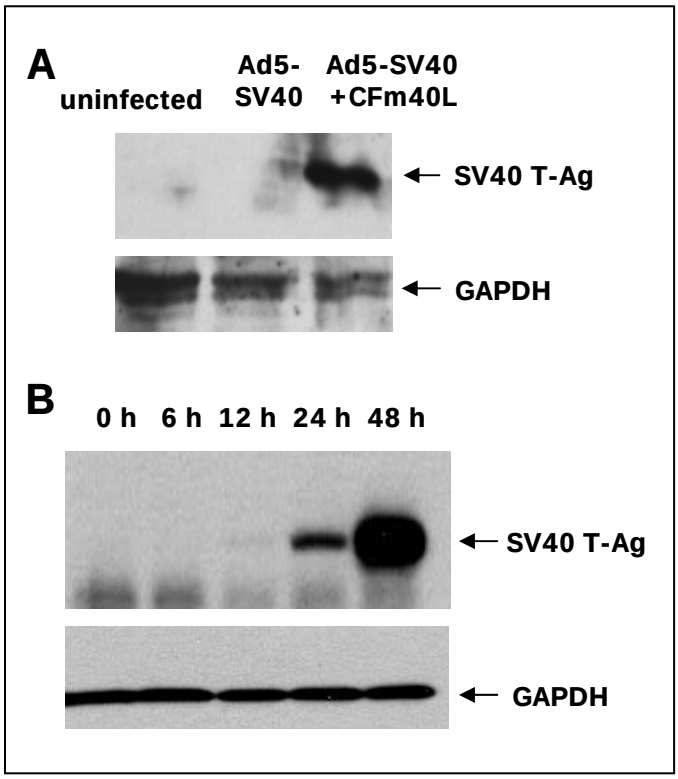


Figure 2. Western blot assay of dendritic cells.
(A) Western Blot assay of untransduced dendritic cells and dendritic cells transduced with untargeted Ad5-SV40 T-Ag and CD40-targeted Ad5-SV40 T-Ag. The expression of SV40 T-Ag is detected only in dendritic cells that are transduced with CD40-targeted Ad5-SV40 T-Ag.
(B) Western Blot assay of dendritic cells transduced with CD40-targeted Ad5-SV40 T-Ag. The expression of SV40 T-Ag is maximal at 48 hrs. post-infection.

can be successfully transduced using a CD40 targeted adenoviral vector with a maximal transgene expression at 48 hours.

RAW 264.7 is a murine macrophage cell line that initially was derived from Balb/c mice infected with Abelson leukemia virus, and is extremely sensitive to lipopolysaccharide (LPS). While studying the interaction of RAW264.7 cells with LPS, it has been observed that a large proportion of LPS-treated RAW 264.7 cells acquire dendritic morphology. In view of these morphological changes, we examined the ability of CD40-targeted Ad vector expressing the SV40 T-Ag [Ad5-(SV40 T-Ag)-CFm40L] to infect RAW 264.7 cells and induce the differentiation into dendritic-like cells. As shown by Western blot analysis (Figure 3A), expression of the SV40 T-Ag transgene occurred only in RAW 264.7 cells that were transduced with the CD40-targeted adenoviral vector (Ad5-SV40 T-Ag). Thus, RAW 264.7 cells were efficiently transduced using a CD40 targeted adenoviral vector, while an untargeted adenoviral vector poorly transduced RAW 264.7 cells. As shown in Figure 3B, RAW 264.7 cells were treated with untargeted and CD40-targeted Ad5-SV40-TAg from 0 to 48 hours, and Western blot analysis was used to determine the level of expression of SV40 T-Ag in the transduced RAW 264.7 cells.

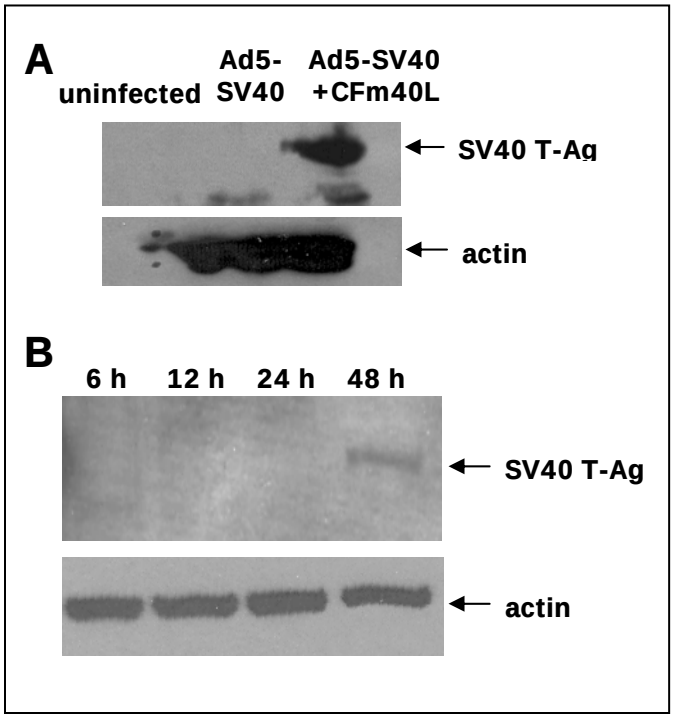
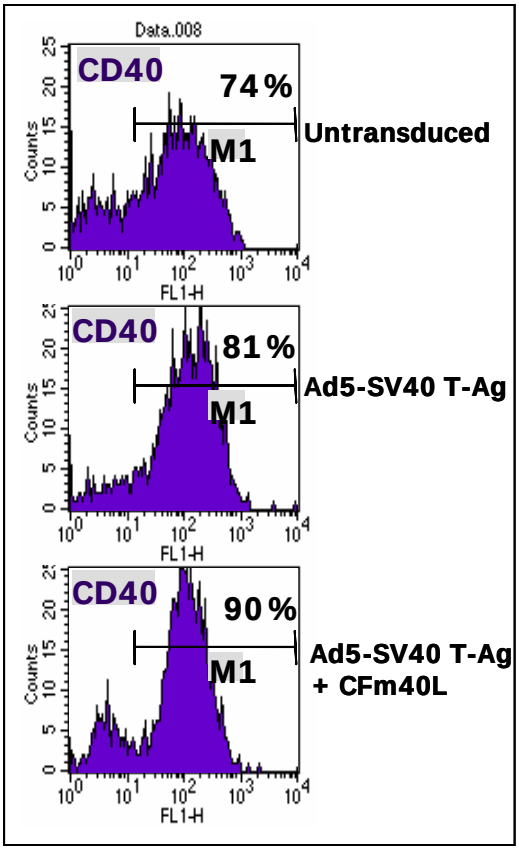
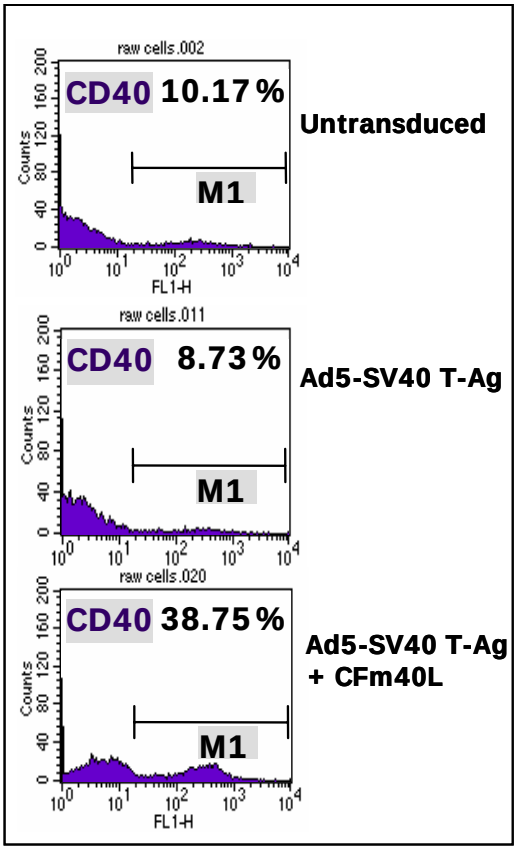


Figure 3. Western blot assay of RAW 264.7 cells.
(A) Western Blot assay of RAW 264.7 cells transduced with CD40-targeted Ad5-SV40 T-Ag. The expression of SV40 T-Ag is detected in the RAW cells at 48 hrs post-infection.
(B) Western Blot assay of RAW 264.7 cells transduced with CD40-targeted Ad5-SV40 T-Ag. The expression of SV40 T-Ag is maximal at 48 hrs post-infection.



Markers	Treatment		
	Untrans-duced	Ad5-SV40 T-Ag	Ad5-SV40 T-Ag + CFm40L
MHC-II	63%	57%	60%
CD40	74%	81%	90%
CD80	6%	19%	25%
CD86	11%	11%	19%
CD11c	67%	54%	62%

Figure 4. Flow cytometric analysis of untransduced dendritic cells and dendritic cells that have been transduced with untargeted Ad5-SV40 T-Ag and CD40-targeted Ad5-SV40T-Ag. The cells were stained with FITC-labeled antibody against activation marker CD40, CD80, CD86, and CD11c.



Markers	Treatment		
	Untrans-duced	Ad5-SV40 T-Ag	Ad5-SV40 T-Ag + CFm40L
MHC-II	0.2%	1.0%	2.8%
CD40	10%	9%	39%
CD80	1.5%	0.3%	0.9%
CD86	0.1%	0.1%	0.1%

Figure 5. Flow cytometric analysis of untransduced RAW 264.7 cells and RAW 264.7 cells that have been transduced with untargeted Ad5-SV40 T-Ag and CD40-targeted Ad5-SV40 T-Ag. The cells were stained with FITC-labeled antibody against activation marker CD40, CD80, CD86, and CD11c.

RAW 264.7 cells transduced with CD40-targeted Ad5-SV40-TAg vector showed increased expression of transgene and expression of co-stimulatory molecules at 48 hours post-infection compared to cells transduced with untargeted Ad5-SV40-TAg vector. These results demonstrate that RAW 264.7 cells can be successfully transduced using a CD40 targeted adenoviral vector with a maximal transgene expression at 48 hours.

To further explore changes in DC maturation, we looked for phenotypic changes that occurred in transduced cells. Molecules involved in antigen presentation, such as CD40, CD80, CD86, CD11c, and MHC class II antigens, are expressed on mature DCs. The uninfected DCs were characterized by flow cytometry and compared to DCs infected with untargeted adenoviral vector Ad5-(SV40 T-Ag) and with CD40-targeted Ad5-(SV40 T-Ag)-CFm40L. As shown in Figure 4, an increase in the cell surface activation marker CD40 from 74% to 81% and in CD80 from 6% to 19% were observed in cells infected with the untargeted vector Ad5-(SV40 T-Ag) compared to uninfected cells. Expression of the CD40 marker was further increased from 81% to 90% in cells infected with the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L compared to cells infected with the untargeted vector Ad5-(SV40 T-Ag). Likewise, expression of the CD80 marker was further increased from 19% to 25% in cells infected with the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L compared to cells infected with the untargeted vector Ad5-(SV40 T-Ag). Expression of the cell surface activation marker CD86 was increased in cells infected with the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L from 11% to 19% compared to uninfected cells or cells infected with the untargeted vector Ad5-(SV40 T-Ag).

To further explore changes in RAW 264.7 cell maturation, we looked for phenotypic changes that occurred in transduced cells. Molecules involved in antigen presentation, such as CD40, CD80, CD86, CD11c, and MHC class II antigens, are expressed on mature RAW 264.7 cells. The uninfected RAW 264.7 cells were characterized by flow cytometry and compared to RAW 264.7 cells infected with untargeted adenoviral vector Ad5-(SV40 T-Ag) and with CD40-targeted Ad5-(SV40 T-Ag)-CFm40L. As shown in Figure 5, an increase in the MHC II marker from 0.2% to 1.0% was observed in cells infected with the untargeted vector Ad5-(SV40 T-Ag) compared to uninfected cells. Expression of the CD40 marker was further increased from 1.0% to 2.8% in cells infected with the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L compared to cells infected with the untargeted vector Ad5-(SV40 T-Ag). Expression of the cell surface activation marker CD40 was increased in cells infected with the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L from 10% to 39% compared to uninfected cells or cells infected with the untargeted vector Ad5-(SV40 T-Ag).

To study DC function, the levels of cytokines and chemokines expressed by DCs and released into the culture medium were quantified by Bio-Plex protein array system (Bio-Rad). Using the mouse 23-plex panel, we measured changes in the cytokines IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, Eotaxin, G-CSF, GM-CSF, and IFN-gamma, and in the chemokines KC, MCP-1 (MCAF), MIP-1alpha, MIP-1beta, RANTES, and TNF-alpha. DCs were infected with untargeted adenovirus Ad5-SV40-TAg vector and with CD40-targeted Ad5-(SV40 T-Ag)-CFm40L. At 24, 48, and 72 hours after infection, media was isolated and changes in cytokine secretion was compared to untreated cells. As shown in Figure 6, infection with CD40-targeted Ad5-(SV40 T-Ag)-CFm40L resulted in a dramatic increase in secretion of the cytokines IL-1alpha, IL-1beta, IL-6, and Il-12 and in the chemokine MIP-1alpha compared to uninfected cells or cells infected with the untargeted vector Ad5-(SV40 T-Ag). Interestingly, secretion of the the chemokine RANTES was induced by infection with either the untargeted vector Ad5-(SV40 T-Ag) or the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L compared to uninfected cells.

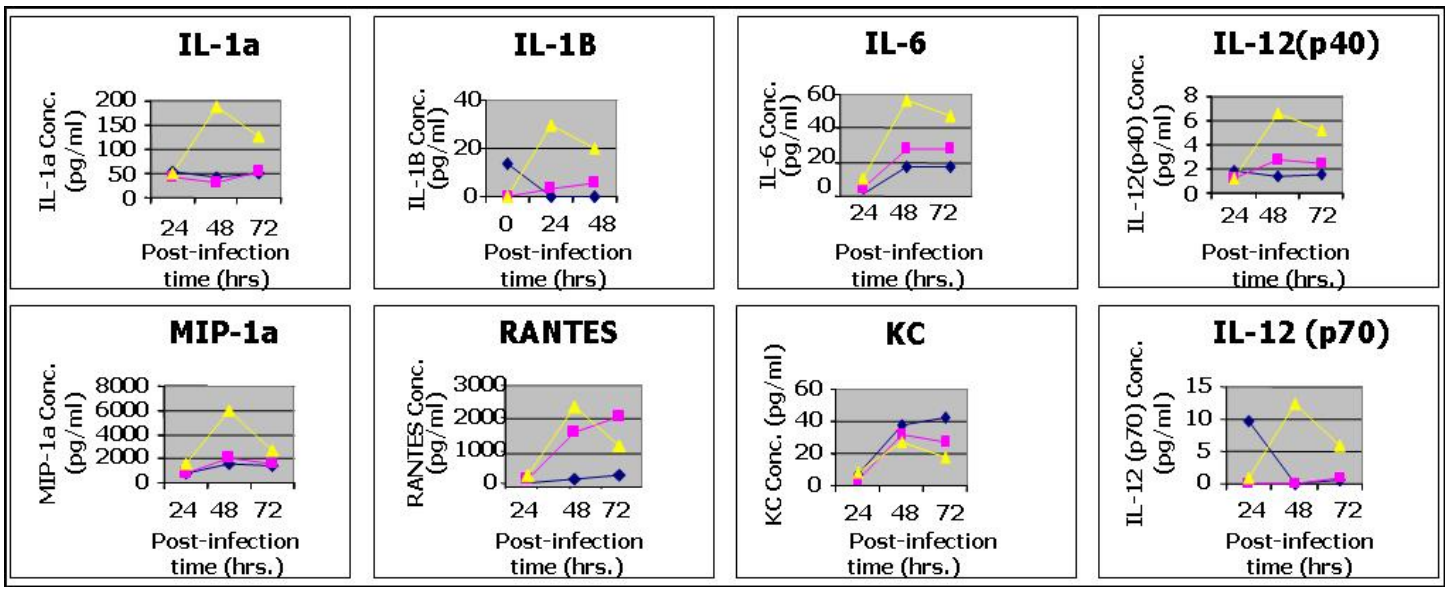


Figure 6. Bio-plex cytokine assay of untransduced dendritic cells and dendritic cells transduced with untargeted Ad5-SV40 T-Ag and CD40-targeted Ad5-SV40 T-Ag. DCs transduced with Ad5-SV40 T-Ag showed increased secretion of cytokines such as IL-1A, IL-1B, IL-6, IL-12 (p40), IL-12 (p70), MIP-1A, RANTES and decreased secretion of cytokine KC compared to untransduced or DCs transduced with untargeted Ad5-SV40 T-Ag.

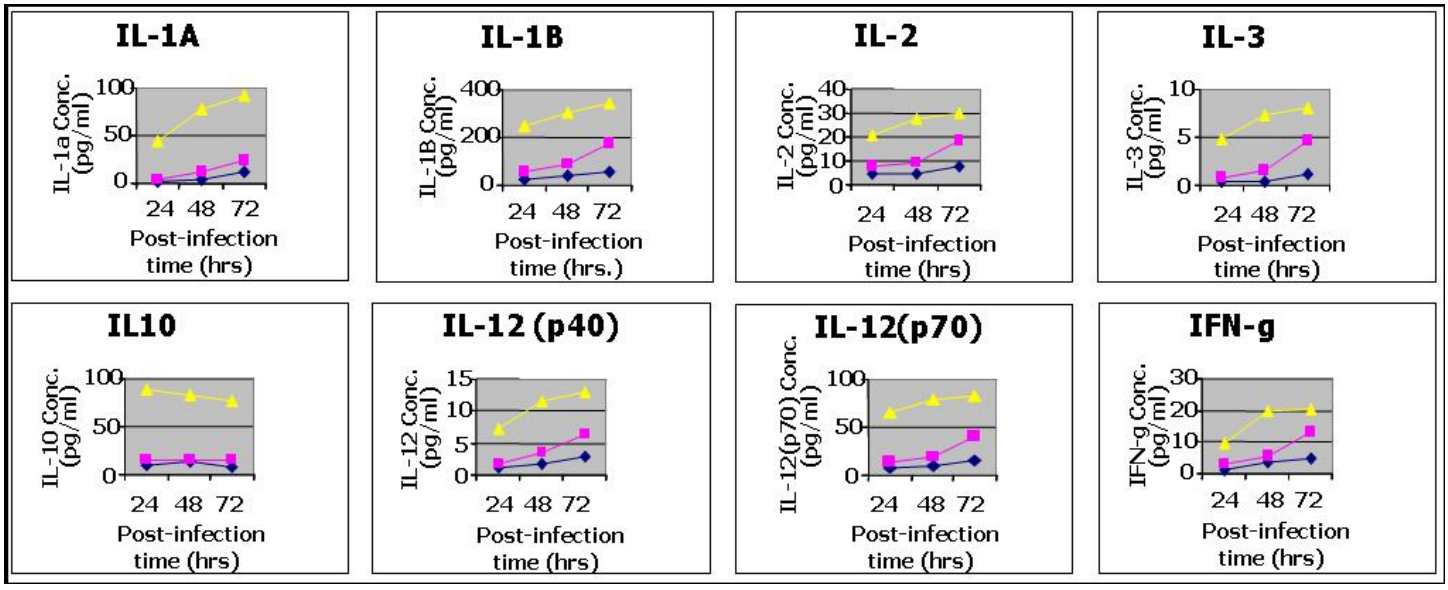


Figure 7. Bio-plex cytokine assay of RAW 264.7 cells and RAW 264.7 cells transduced with untargeted Ad5-SV40 T-Ag and CD40-targeted Ad5-SV40 T-Ag. RAW 264.7 cells transduced infected with CD40-targeted Ad5-SV40 T-Ag showed increased secretion of cytokines compared to untransduced RAW 264.7 cells or cells transduced with untargeted Ad5-SV40 T-Ag alone. The relative increase in secretion of cytokines was maximal at 48 hrs. post-infection.

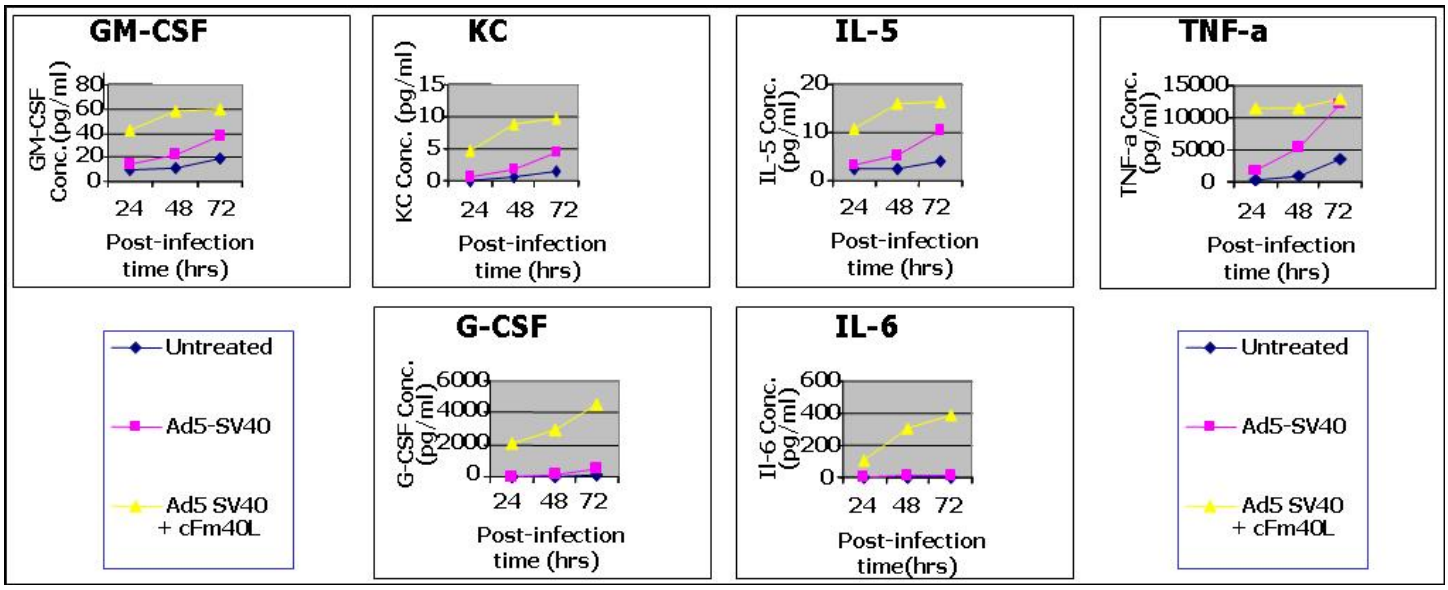


Figure 8. Bio-plex cytokine assay of RAW 264.7 cells and RAW 264.7 cells transduced with untargeted Ad5-SV40 T-Ag and CD40-targeted Ad5-SV40 T-Ag. RAW 264.7 cells transduced with CD40-targeted Ad5-SV40 T-Ag showed increased secretion of cytokines compared to untransduced RAW 264.7 cells or cells transduced with untargeted Ad5-SV40 T-Ag. The relative increase in secretion of cytokines was maximal at 48 hrs. post-infection.

To study RAW 264.7 cell function, the levels of cytokines and chemokines expressed by RAW 264.7 cells and released into the culture medium were quantified by Bio-Plex protein array system (Bio-Rad). Using the mouse 23-plex panel, we measured changes in the cytokines IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, Eotaxin, G-CSF, GM-CSF, and IFN-gamma, and in the chemokines KC, MCP-1 (MCAF), MIP-1alpha, MIP-1beta, RANTES, and TNF-alpha. RAW 264.7 cells were infected with untargeted adenovirus Ad5-SV40-TAg vector and with CD40-targeted Ad5-(SV40 T-Ag)-CFm40L. At 24, 48, and 72 hours after infection, media was isolated and changes in cytokine secretion was compared to untreated cells. As shown in Figures 7 and 8, infection with CD40-targeted Ad5-(SV40 T-Ag)-CFm40L resulted in a dramatic increase in secretion of the cytokines IL-1alpha, IL-1beta, IL-2, IL-3, IL-5, IL-6, IL-10, IL-12, G-CSF, and GM-CSF and in the chemokine KC compared to uninfected cells or cells infected with the untargeted vector Ad5-(SV40 T-Ag).

To study the effect of CD40-targeted Ad transduction on DC-mediated CTL activation, we made use of the untargeted adenovirus vector Ad5-(SV40 T-Ag) and the CD40-targeted adenovirus vector Ad5-(SV40 T-Ag)-CFm40L. In the experiment shown in Figures 9 and 10, B6C3F1 mice were immunized with 1 x 10^8 i.f.u. of untargeted or CD40 targeted adenovirus vector. This immunization was repeated at 14 days, and at 24 days from initiation of the immunization regimen, the mice were sacrificed and the spleens were removed. Splenocytes from the mice were re-stimulated for 6 days with SV40 T-Ag peptides. Following re-stimulation, a classic ^{51}Cr release CTL assay was performed with the stimulated CTL as effector cells (E) and non-specific CMT.64 cells as a negative control (Figure 9) or MOVCAR-2 cells as a specific target (Figure 10) (T) at the indicated E:T ratios. Cytotoxicity was measured by ^{51}Cr release from MOVCAR-2 or CMT.64 target cells. Splenocytes from mice in the vaccination

group receiving either untargeted adenovirus vector Ad5-(SV40 T-Ag) or the vaccination group receiving CD40-targeted adenovirus vector Ad5-(SV40 T-Ag)-CFm40L exhibited non-specific lysis against CMT.64 cells (Figure 9). However, splenocytes from mice in the vaccination group in the vaccination group receiving either untargeted adenovirus vector Ad5-(SV40 T-Ag) or the vaccination group receiving CD40-targeted adenovirus vector Ad5-(SV40 T-Ag)-CFm40L also showed no acquisition of killing activity (Figure 10). The differences between control group and the other two groups were statistically significant at E:T ratios of 100:1, 50:1, and 25:1 ($P < 0.01$). IFN-γ induces the expression of several components of the antigen-processing machinery, leading to enhanced presentation of peptides in the context of HLA class I molecules on the cell surface. This facilitates the T cell receptor–mediated (TCR-mediated) recognition of target cells by CD8+ T cells. Therefore we tested the effect of pre-treatment with an adenoviral vector expressing the murine interferon-gamma (Ad-IFN-γ) to enhance CTL lysis of target cells. As shown in Figure 10, CTL reactivity was significantly enhanced in MOVCAR-2 cells, which were pre-treated for 24 hours with a 100:1 multiplicity of infection of Ad-IFN-γ compared to untreated MOVCAR-2 cells. Importantly, specific lysis was not observed in the negative control CMT.64 cells despite pre-treatment with Ad-IFN-γ (Figure 9).

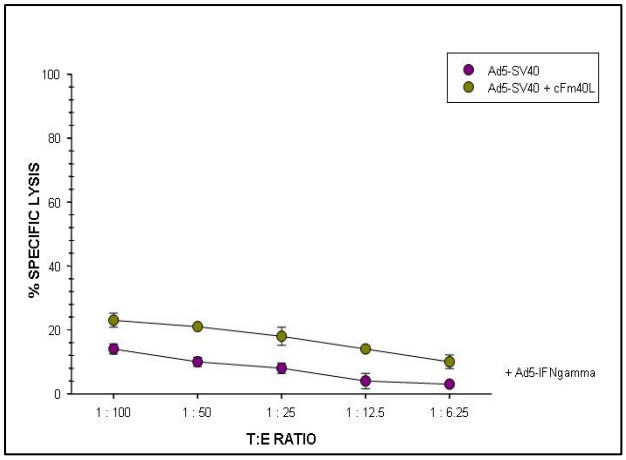


Figure 9. A standard 4-hour ^{51}Cr-release assay against CMT.64 cells (negative control).

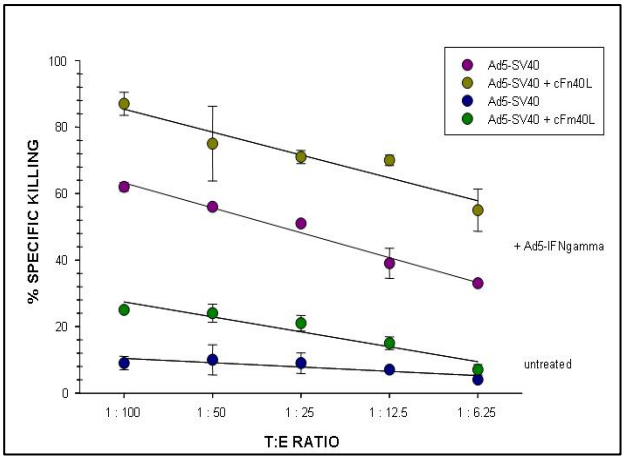


Figure 10. A standard 4-hour ^{51}Cr release assay against target cells, MOVCAR-2 (Ad5-IFN-γ treated as well as untreated). The T-cells that were obtained from mice that were treated with Ad5-SV40 + CFm40L showed higher cytolytic activity against the target cells compared to the T-cells obtained from mice that were treated with Ad5-SV40 alone.

The results studies clearly demonstrate the need for an immunological adjuvant, as a dramatic improvement of CTL lysis was observed in MOVCAR-2 cells expressing SV40 T-Ag that were treated with IFN-γ. We have also found that transduction of MOVCAR-2 cells with an Ad vector expressing interferon-gamma (Ad-IFN-γ) resulted in up-regulation of MHC class I molecule and TAP protein expression (data not shown). Thus, up-regulation of these proteins was necessary for efficient antigen presentation in RM-1 target cells. Down regulation of MHC class I molecule expression, and lack of co-stimulatory molecules are defects that render tumor cells

invisible to the immune system. Re-establishment of these signals by delivery of cytokines, co-stimulatory molecules, and even MHC antigens have all been approaches used for cancer immunotherapy.

Key Research Accomplishments

1. Genetic Immunotherapy Targeted to Antigen Presenting Cells Using a CD40-Targeted Adenoviral Vector (2005) Disha A. Mody, Larry Smart, Yoshinobu Odaka, Xiao L. Li, Cigdem E. Yilmaz, Alexander V. Pereboev, J. Michael Mathis American Society of Gene Therapy Eighth Annual Meeting. Abstract 274.
2. Dendritic Cell-Based Genetic Immunotherapy for Prostate Cancer Briana J. Williams, Nikolay Korokhov, Susan Boling, Linda Li, Michael Mathis, David T. Curiel American Society of Gene Therapy Eighth Annual Meeting. Abstract 316.
3. Genetic Immunotherapy Targeted to Antigen Presenting Cells Using a CD40-Targeted Adenoviral Vector (2005) J.M. Mathis, D.A. Mody, L. Smart, Y. Odaka, X.L. Li, C.E. Yilmaz, D.T. Curiel, and A.V. Pereboev Thirteenth Annual Congress of the European Society of Gene Therapy. Abstract 274.
4. Changes in Maturation Profiles of Dendritic Cells Transduced with a CD40-Targeted Adenoviral Vector (2006) Disha A. Mody, Alexander V. Pereboev, Don A. Sibley, David T. Curiel, J. Michael Mathis American Society of Gene Therapy Ninth Annual Meeting. Abstract 836.

Reportable Outcomes

Task 1

- We have characterized the phenotypic changes in vitro in isolated dendritic cells and RAW 264.7 cells after infection with a CD40-targeted Ad vector compared to an untargeted Ad.
- We have demonstrated dramatically enhanced gene transfer using the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L vector
- Infection with the CD40-targeted Ad5-(SV40 T-Ag)-CFm40L vector induced phenotypical maturation and up-regulated cytokine expression.

Task 2

- We have established and characterized a syngeneic immunotherapy model of ovarian cancer using the MOVCAR-2 mouse ovarian tumor cell line.
- We have determined the ability of dendritic cells infected with Ad5-(SV40 T-Ag)-CFm40L ex vivo and transferred to host animals, to activate CTLs in vivo against MOVCAR cells.

Conclusions

- Western blot analysis of the antigen presenting cells (APCs) showed that the APCs can be successfully transduced using a CD40 targeted adenoviral vector. The expression of the transgene, SV40 T-Ag, as shown by the western blot analysis, occured only in cells that have been transduced with a CD40-targeted adenoviral vector (Ad5-SV40 T-Ag).
- Flow cytometric analyses of the APCs showed an increase in the expression of the cell surface activation marker CD40 in dendritic cells and untreated RAW cells that have been transduced

with the CD40-targeted adenoviral vector compared to the cells that were untransduced or transduced with the adenoviral vector alone.

- Bio-plex cytokine assay showed an increased secretion of cytokines by the APCs that have been transduced with the CD40-targeted adenoviral vector compared to the cells that were untransduced or transduced with adenoviral vector alone.
- These results demonstrate that APCs can be successfully transduced using a CD40-targeted adenoviral vector and these transduced APCs show promise as candidates for use in tumor immunotherapy.
- We have demonstrated that direct immunization of mice in situ with a CD40-targeted Ad together with IFN-g treatment of target cells is effective in inducing an antigen-specific CTL response to SV40 T-Ag.

References

Appendices

American Society of Gene Therapy Ninth Annual Meeting. Abstract 836.

Changes in Maturation Profiles of Dendritic Cells Transduced with a CD40-Targeted Adenoviral Vector

Disha A. Mody, Alexander V. Pereboev, Don A. Sibley, David T. Curiel, J. Michael Mathis Cellular Biology and Anatomy, LSU Health Sciences Center, Shreveport, LA; Division of Human Gene Therapy, University of Alabama at Birmingham, Birmingham, AL

Introduction: Adenovirus (Ad)-mediated transduction of dendritic cells (DCs) is inefficient because of the lack of the primary Ad receptor, CAR. CD40 is a surface marker expressed by DCs that plays a crucial role in their maturation and subsequent stimulation of T cells. DC infection with Ad targeted to the CD40 results in increased gene transfer. Recently, we characterized a new model using a mouse ovarian carcinoma cell line (MOVCAR) that expresses the SV40 large T-Ag and forms tumors in syngeneic immunocompetent B6C3F1 mice. The SV40 large T-Ag is highly immunogenic, inducing both antibody and cytotoxic T lymphocyte (CTL) responses. Since this antigen is synthesized in MOVCAR cells, the SV40 large T-Ag is an attractive candidate as a model system for the development of a DC-targeted cancer vaccine. We describe the further characterization of the CD40-targeting approach using an adapter molecule that bridges the fiber of the Ad5 to CD40 on mouse DC. This adapter molecule, CFm40L, consists of the ectodomain of CAR genetically linked via a trimerization motif to the extracellular domain of mouse CD40 ligand. We have demonstrated that DCs transduced with a CD40-targeted Ad vector expressing SV40 T-Ag (Ad5-SV40-TAg) showed an increased expression of cell surface activation markers and were effective in inducing an antigen-specific CTL response. To examine CD maturation induced by transduction with the CD40-targeted Ad vector, we examined changes in a panel of cytokines and chemokines. In addition, we examined the kinase signalling pathways involved in cytokine regulation.

Methods: We used a Bio-Plex assay was performed on supernatants from untransduced DCs and DCs transduced with untargeted Ad and CD40-targeted Ad5 to determine changes in production of a panel of 23 cytokines and chemokines at 24, 48, and 72 hours post infection. We used a Kinexus phospho-antibody screening system on cell lysates from untransduced DCs and DCs transduced with untargeted Ad and CD40-targeted Ad to examine changes in phosphorylation / activation of 34 different protein phospho-kinases

Results: DCs transduced with a CD40-targeted Ad showed increased secretion of the cytokines IL-1A, IL-1B, IL-6, and IL-12, as well as chemokines MIP-1A and RANTES compared to untransduced DCs or DCs transduced with untargeted Ad. Similarly, DCs transduced with a CD40-targeted Ad showed a decreased secretion of cytokine KC. DCs transduced with a CD40-targeted Ad showed increased phosphorylation of MEK1, Erk1, Erk, and STAT1(T385), and decreased phosphorylation of Akt1, compared to untransduced DCs or DCs transduced with untargeted Ad. In contrast, DCs transduced with a CD40-targeted Ad showed an inhibition of Src, Gsk3a, Gsk3b, and STAT1(S727) phosphorylation compared to DCs transduced with untargeted Ad.
Conclusions: We demonstrated Ad-mediated CD40-targeted gene transfer to murine DCs using an adapter molecule CFm40L promotes DC maturation with induction of a complex signaling cascade accompanied by characteristic changes in cytokine production.
Keywords: Adenovirus; Immunotherapy; Targeted Gene Expression

Thirteenth Annual Congress of the European Society of Gene Therapy. Abstract 110.

Genetic Immunotherapy Targeted to Antigen Presenting Cells Using a CD40-Targeted Adenoviral Vector

J.M. Mathis[1], D.A. Mody[1], L. Smart[1], Y. Odaka[1], X.L. Li[1], C.E. Yilmaz[1], D.T. Curiel[2], and A.V. Pereboev[2]

[1]Gene Therapy Program, LSU Health Sciences Center, Shreveport, LA, 71130 USA
[2]Division of Human Gene Therapy, University of Alabama at Birmingham, Birmingham, AL 35294-2172, USA

Dendritic cells (DCs) capture, process and present antigens in association with MHC class I and class II molecules to naive CD8+ cytotoxic and CD4+ helper T cells. Through this, specific cytotoxic T cells are activated, and recognize a target cell and kill it. This study was to determine the transduction efficiency of DCs using a CD40-targeted adenoviral vector expressing a tumor antigen. Recently, we characterized a new model using a mouse ovarian carcinoma cell line (MOVCAR) that expresses the SV40 large T-Ag and forms tumors in syngeneic mice. The SV40 large T-Ag is highly immunogenic, inducing both antibody and cytotoxic T lymphocyte (CTL) responses. Since this antigen is synthesized in MOVCAR cells, the SV40 large T-Ag is an attractive candidate as a model system for the development of a DC-targeted cancer vaccine. We hypothesize that transduction of DCs in vitro using a CD40-targeted Ad5 vector expressing SV40 T-Ag (Ad5-SV40-TAg) will result in a high level of transgene expression, and be effective in inducing an antigen-specific CTL response. To target Ad5-SV40-TAg to DCs, we utilized a recombinant adapter protein consisting of extracellular portion of the native adenovirus receptor, CAR, fused to a trimerization motif from T4 fibritin protein, and linked to the extracellular domain of the mouse CD40 ligand. DCs were treated with untargeted and CD40-targeted Ad5-SV40-TAg using increasing multiplicities of infection. Western blot analysis was used to determine the level of expression of SV40 T-Ag in the transduced DCs, and flow cytometric analysis was used to determine the changes in the expression of cell surface markers. Cells transduced with CD40-targeted Ad5-SV40-TAg vector showed increased expression of transgene and expression of co-stimulatory molecules at 48 hours post-infection compared to

cells transduced with untargeted Ad5-SV40-TAg vector. These results demonstrate that DCs can be successfully transduced using a CD40 targeted adenoviral vector and that transduced DCs show activation.

American Society of Gene Therapy Eighth Annual Meeting. Abstract 316.

Dendritic Cell-Based Genetic Immunotherapy for Prostate Cancer

Briana J. Williams, Nikolay Korokhov, Susan Boling, Linda Li, Michael Mathis, David T. Curiel. Urology and Cell Biology/Anatomy, LSU Health Sciences Center, Shreveport, LA; VectorLogics, Inc., Birmingham, AL; Division of Human Gene Therapy, University of Alabama-Birmingham, Birmingham, AL

Human prostate tumor vaccine and gene therapy trials using ex vivo methods to prime dendritic cells (DCs) with prostate specific membrane antigen (PSMA) have been somewhat successful, but heretofore the lengthy ex vivo manipulation of DCs has limited the utility of this approach. Our goal was to improve upon DC vaccination with tumor antigens by delivering PSMA via a CD40-targeted adenoviral (Ad) vector expressing PSMA directly to DCs in situ as an efficient means for activation and antigen presentation to T-cells. We initially developed a mouse model of prostate cancer by generating several clonal derivatives of the mouse RM-1 prostate cancer cell line that express high levels of human PSMA (RM-1-PSMA). We then tested the efficacy of the CD40-targeted Ad-PSMA vector to stimulate a cytotoxic T-cell (CTL) response in vivo. In order to maximize antigen presentation in target cells, we induced both MHC class I and Tap protein expression in RM-1 cells by treatment with interferon-gamma (IFN-g). Using RM-1-PSMA cells pretreated with IFN-g as target cells, we demonstrated that DCs infected ex vivo with Ad-PSMA, as well as those infected by direct intraperitoneal injection of the CD40-targeted Ad-PSMA resulted in high levels of tumor-specific CTL responses, compared to responses with a control adenovirus (Ad-luc1). Thus, we have demonstrated that direct immunization of DCs in situ with a CD40-targeted Ad together with IFN-g treatment of target cells is effective in inducing an antigen-specific CTL response to PSMA. We have initiated animal studies to test the efficacy of the CD40-targeted PSMA vector versus a non-targeted vector with and without stimulation by IFN-g in protecting against a lethal RM-1-PSMA tumor challenge. At day 14 post-tumor inoculation, only those animals treated with the CD40-targeted adenovirus and IFN-g were resistant to challenge with RM-1-PSMA tumor cells. Our next aim is to determine the lowest effective dosage of targeted vector and the optimal combination of cytokine/chemokine stimulation to protect against tumor challenge as well as test the efficacy of the targeted vector strategy in a minimal residual disease model. The in vitro studies coupled with the syngeneic tumor survival model will allow us to systematically analyze this approach and generate data directly applicable for a human Phase I clinical trial.

Keywords: Cancer Gene Therapy; Vaccines; Cancer Immunology

American Society of Gene Therapy Eighth Annual Meeting. Abstract 274.

Genetic Immunotherapy Targeted to Antigen Presenting Cells Using a CD40-Targeted Adenoviral Vector

Disha A. Mody, Larry Smart, Yoshinobu Odaka, Xiao L. Li, Cigdem E. Yilmaz, Alexander V. Pereboev, J. Michael Mathis. Cellular Biology and Anatomy, LSU Health Sciences Center, Shreveport, LA; Division of Human Gene Therapy, University of Alabama at Birmingham, Birmingham, AL

Introduction: Antigen Presenting Cells (APCs) specialize in capturing foreign antigens, displaying them on their cell surface along with MHC molecules to the lymphocytes and providing activation signals for their differentiation and proliferation. The main cell types that serve as APCs are dendritic cells and macrophages. They can capture, process and present antigens in association with MHC class I and class II molecules to naïve CD8+ (cytotoxic) and CD4+ (helper) T lymphocytes, respectively. Through this process, specific cytotoxic T lymphocytes for that antigen are activated and they can recognize a target cell and kill it. The goal of this study was to determine the transducion efficiency of APCs using a CD40-targeted adenoviral vector expressing a tumor antigen. A second goal was to characterize transduced APCs to determine if APCs are suitable for use in tumor immunotherapy.

Recently, we characterized a new model using a mouse ovarian carcinoma cell line (MOVCAR) that expresses the SV40 large T-Ag and forms tumors in syngeneic immunocompetent B6C3F1 mice. The SV40 large T-Ag is highly immunogenic, inducing both antibody and cytotoxic T lymphocyte (CTL) responses. Since this antigen is synthesized in MOVCAR cells, the SV40 large T-Ag is an attractive candidate as a model system for the development of a DC-targeted cancer vaccine. We hypothesize that transduction of APCs *in vitro* using a CD40-targeted Ad vector expressing SV40 T-Ag (Ad5-SV40-TAg) will result in a high level of transgene expression, and be effective in inducing an antigen-specific CTL response in vivo.

Methods: Primary cultures of mouse dendritic cells and cultures of the mouse RAW 264.7 cell line treated with lipopylysaccharide (LPS) were used as sources of APCs. To target Ad5-SV40-TAg to APCs, we utilized a recombinant adapter protein consisting of extracellular portion of the native adenovirus receptor, CAR, fused to a trimerization motif of a 71 amino acid domain from the pactriophage T4 fibritin protein, and linked to the extracellular domain of the mouse CD40 ligand. The APCs were treated with untargeted and CD40-targeted Ad5-SV40-TAg using increasing multiplicities of infection. Western blot analysis was used to determine the level of expression of the SV40 T-Ag in the transduced APCs, and flow cytometric analysis was used to determine the changes in the expression of the cell surface markers on the transduced APCs.

Results: Cells that were transduced with the CD40-targeted Ad5-SV40-TAg vector showed increased expression of transgene at 48 hours post-infection compared to cells tranduced with untargeted Ad5-SV40-TAg vector. In addition, APCs transduced with the CD40-targeted Ad5-SV40-TAg vector also showed increased expression of co-stimulatory molecules compared to untransduced APCs or to APCs tranduced with untargeted Ad5-SV40-TAg.

Conclusions: The results demonstrate that the APCs can be successfully transduced using a CD40 targeted adenoviral vector and that transduced APCs show increased expression of cell surface activation markers.

Keywords: Immunotherapy; Adenovirus; Cancer Gene Therapy